Urine flow cytometry can rule out urinary tract infection, but cannot identify bacterial morphologies correctly

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Urine flow cytometry can rule out urinary tract infection, but cannot identify bacterial morphologies correctly

N. Geerts [a,*], A.R. Jansz [b], K.J.M. Boonen [a,1], R.P.W.F. Wijn [c], E.L. Koldewijn [c], A.K. Boer [a], V. Scharnhorst [a]

[a] Clinical Laboratory, Catharina Hospital Eindhoven, Michelangelolaan 2, 5623 EJ Eindhoven, The Netherlands
[b] Department of Medical Microbiology, Laboratory for Pathology and Medical Microbiology (PAMM), De Run 6250, 5504 DL Veldhoven, The Netherlands
[c] Department of Urology, Catharina Hospital Eindhoven, Michelangelolaan 2, 5623 EJ Eindhoven, The Netherlands

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ABSTRACT

The diagnosis of urinary tract infection (UTI) by urine culture is a time-consuming and costly procedure. Usage of a screening method, to identify negative samples, would therefore affect time-to-diagnosis and laboratory cost positively. Urine flow cytometers are able to identify particles in urine. Together with the introduction of a cut-off value, which determines if a urine sample is subsequently cultured or not, the number of cultures can be reduced, while maintaining a low level of false negatives and a high negative predictive value. Recently, Sysmex developed additional software for their urine flow cytometers. Besides measuring the number of bacteria present in urine, information is given on bacterial morphology, which may guide the physician in the choice of antibiotic. In this study, we evaluated this software update. The UF1000i classifies bacteria into two categories: 'rods' and 'cocci/mixed'. Compared to the actual morphology of the bacterial pathogen found, the 'rods' category scores reasonably well with 91% chance of classifying rod-shaped bacteria correctly. The 'cocci/mixed' category underperforms, with only 29% of spherical-shaped bacteria (cocci) classified as such. In its current version, the bacterial morphology software does not classify bacteria, according to their morphology, well enough to be of clinical use in this study population.

1. Introduction

Urinary tract infections (UTI) are the most common infections in both hospitalized patients and the general community [1]. The gold standard to diagnose a UTI is a positive bacterial culture. Unfortunately, bacterial culture is a time-consuming and costly procedure. Moreover, when bacterial culture is used for the diagnoses of UTI, results are not readily available for the physician, leading to a delay in proper care. This problem is often circumvented by diagnosing on clinical manifestation alone. Symptoms like fever, pain, or a burning sensation during micturition and complaints of urgency or frequency are considered specific enough to diagnose a UTI. However, diagnosing on symptoms alone will overestimate the presence of UTI by 33% [2]. This overestimation leads to a large amount of unnecessarily prescribed antibiotics.

Usage of a screening method, to identify samples that do not have to be cultured, would not only affect time-to-diagnosis and laboratory cost positively, it will also prevent the unnecessary prescription of antibiotics. For this reason, automated methods for urine analysis have been developed. The Sysmex urine flow cytometers are able to identify particles in urine by scattering and fluorescence (after staining). The feasibility of using urine flow cytometry to reduce the number of cultures, by screening for urine samples that will lead to no, or no significant growth has been analyzed [3–7]. Indeed, the introduction of a cut-off value, which determines if urine is subsequently cultured or not, can reduce the number of cultures, while maintaining a low level of false negatives and a high negative predictive value. In parallel with the reduction in cultures, antibiotic prescriptions might be reduced, as screening results can be available within 30 min, in contrast to at least a day for culturing.

Recently, Sysmex developed additional software for their urine flow cytometers. After installation of this software upgrade the flow cytometer can give an indication on bacterial morphology in addition to the number of bacteria present within the urine. UTIs are often the result of an infection with rod-shaped bacteria (e.g. *Escherichia Coli*, *Proteus Mirabilis*) [8]. Most of these are considered uncomplicated UTIs and can be treated with a standard antibiotic. In contrast, UTIs as a result of an infection with spherical-shaped (cocci) bacteria (e.g. *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus agalactiae*) are deemed (more) complicated. Treatments of these types of UTI

* Corresponding author at: Catharina Hospital Eindhoven, Clinical Laboratory, P.O. box 1350, 5602 ZA Eindhoven, The Netherlands.
E-mail addresses: nienke.geerts@catharinaziekenhuis.nl (N. Geerts), a.jansz@pamm.nl (A.R. Jansz), kboonen@amphia.nl (K.J.M. Boonen), rob.wijn@mumc.nl (R.P.W.F. Wijn), evert.koldewijn@catharinaziekenhuis.nl (E.L. Koldewijn), arjen-kars.boer@catharinaziekenhuis.nl (A.K. Boer), volkher.scharnhorst@catharinaziekenhuis.nl (V. Scharnhorst).
[1] Current address: Clinical Laboratory, Amphia Hospital, Molengracht 21, 4818 CK Breda, The Netherlands.

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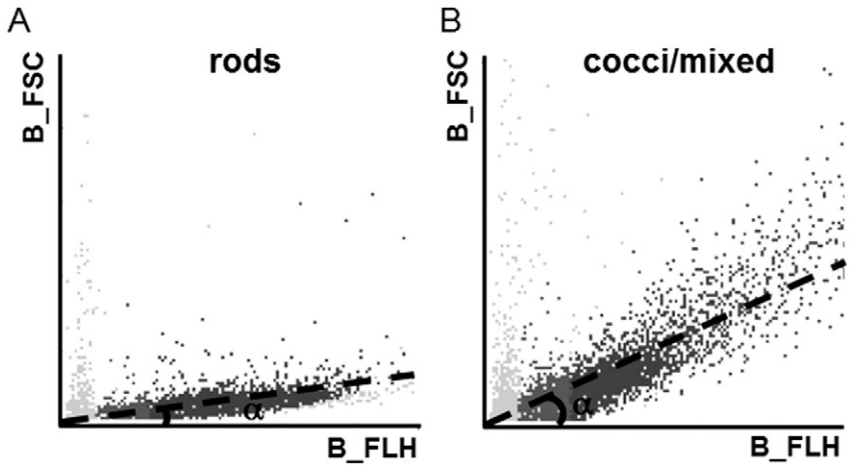

Fig. 1. The UF1000i uses the scattergram obtained from the forward (cell volume) and side scatter (fluorescence), of each bacterium passing its laser beam, to report the bacterial morphology for each urine sample analyzed. Depending on the angle of the scatter cloud with the *x*-axis the bacteria are classified as either 'rods' (A) or 'cocci/mixed' (B).

usually require a more aggressive type of antibiotic [9,10]. Therefore, including information on bacterial morphology, on top of the number of bacteria found, may guide the physician in the choice of antibiotic.

Bacterial morphology indication is the latest feature of the Sysmex urine flow cytometer. Hereto, the UF1000i detects the forward (cell volume) and side scatter fluorescence of each bacterium passing its laser beam. These results are plotted in a scattergram. Initially, it was observed that gram negative bacteria resulted into a different scatter pattern than gram positive bacteria [11]. Later, this was adjusted to bacterial morphology rather than gram staining. To classify bacterial morphology, a straight line is drawn through the middle of the obtained scatter cloud: the angle with the *x*-axis determines if the bacteria found are classified as 'rods' (<30°) or 'cocci/mixed' (>30°) (Fig. 1).

In this study, we evaluated the latest feature of the Sysmex UF-1000i: the ability to correctly indicate the bacterial morphology of the pathogen found. First, the UF1000i morphology indication is compared to the actual morphology of the bacterial species identified in the urine culture, to assess if bacteria are classified correctly as either 'rods' or 'cocci/mixed'. To get insight on the clinical usefulness of this new feature, screening and culture results were analyzed to determine if foreknowledge on bacterial morphology can lead to changes in what antibiotic to prescribe.

2. Materials and methods

2.1. Evaluating bacterial morphology software

To evaluate the bacterial morphology software 662 urine samples from our hospital population were initially included (354 from outpatients (53%), 308 from hospitalized patients (47%); 265 male (40%), 397 female (60%)). Most patients originated from the urology department (57 %), followed by Internal Medicine (18%). The remaining samples (25%) originated from all other departments combined. All urines were cultured and had their number of bacteria/μl determined on the urine flow cytometer UF1000i[2] (Sysmex Benelux, Etten-Leur, The Netherlands). The final analysis is done on those urines that had a bacteria count above the cut-off value of 200 bacteria/μl ($N = 510$).

2.2. UF1000i analysis

Sysmex UF1000i performed an analysis of particles in (midstream) urine by flow cytometry. This flow cytometer has two chambers, where diluted urine can be incubated with specific dye and lysis reagents. One chamber is used solely for bacteria, the other for all other particles (erythrocytes, leukocytes, casts, etc.). After staining, the samples are transported to a flow cell where they are analyzed with the use of a semiconductor laser, and characterized by forward scatter, side scatter, and fluorescence. In this study, only the bacteria count and subsequent morphology indication are used.

2.3. Microbiological analysis

The UriSwab was sent to the laboratory for medical microbiology (PAMM laboratories). The UriSwab was centrifuged, and after gram staining, 10 μl was plated on a Brilliance UTI Clarity Agar (Oxoid, Basingstoke, UK) as well as a blood agar plate containing 5 μg/ml colistin and 2 mg/ml aztreonam (CAP agar). Both plates were examined for growth after 18–24 h (incubation at 35 °C). Grown colonies were identified by color or VITEK2® (bioMerieux, France) if necessary. A minimum level of bacteriuria demonstrating a UTI is not defined in scientific literature. Positive cultures are defined by thresholds ranging from 10^3 to 10^5 cfu/ml urine, depending on the species found [2]. In this study a positive culture is not defined by its cfu/ml. Instead, the inclusion of an antibiogram by the microbiologist dermines if a culture is deemed positive for an UTI. This expert opinion considers both the cfu/ml and the type of pathogen found.

3. Results

3.1. Evaluating bacterial morphology software: culture results.

To evaluate the bacterial morphology software 662 urine samples were initially analyzed. Within our hospital we have implemented two types of requests for UTI analysis. When "exclusion UTI" is requested, the UriSwab is only sent for culture if the bacterial count exceeds the cut-off value of 200 bacteria/μl. When "bacterial count" is requested, UriSwabs are always sent to the microbiological laboratory for culture [3]. Currently, 63% of UTI analyses are "bacterial count" requests (73% of all UriSwabs cultured are negative; 3 urine samples with a positive culture had a bacterial count < 200 bacteria/μl (false negative; 1× *Streptococcus agalactiae* — 145 bac/μl; 1× *Enterococcus faecium* — 139 bac/μl; 1× *Klebsiella pneumoniae* — 24 bac/μl, with a comment of possible additional growth in container)). The remaining 37% are "exclusion UTI" requests (57% of all UriSwabs cultured are negative). Urines with a bacterial count above 200 bacteria/μl were included in the final bacterial morphology analysis (510 urine samples; 48% of UriSwabs cultured are positive). The 'rods' indication was reported for 280 urine samples, resulting in 171 positive cultures (61%), the 'cocci/

[2] Reagents UF1000i supplied by Sysmex.

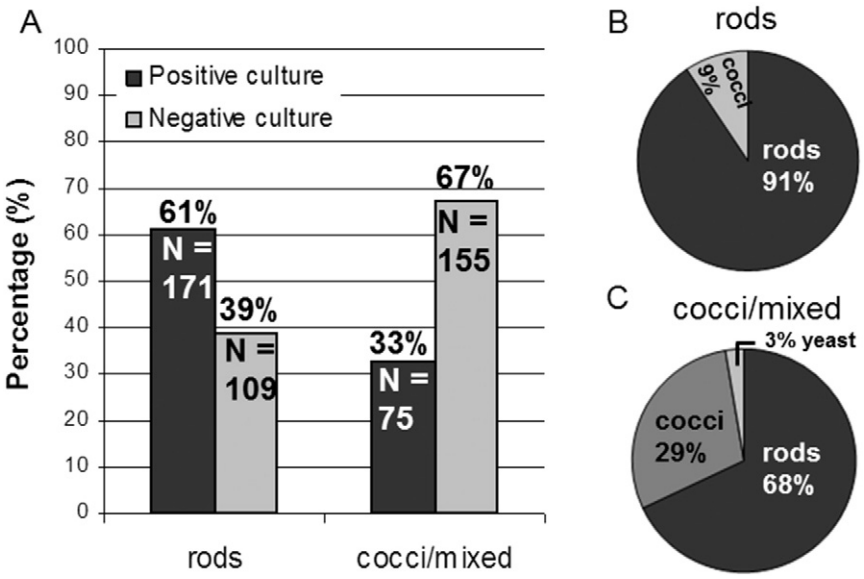

Fig. 2. Culture results. A. Percentage of positive and negative cultures per bacteria morphology as indicated by the UF1000i shape indication. B. UF1000i bacterial morphology indication compared to the bacterial shape of the species found in positive cultures if the UF1000i indicated 'rods'. C. When the UF1000i indicated 'cocci/mixed'.

mixed' indication was reported for 230 urine samples resulting in 75 positive cultures (33%, Fig. 2A).

3.2. Evaluating bacterial morphology software: morphology classification

The bacterial morphology reported by the UF1000i was compared to the actual shape of the species found in positive cultures. Fig. 2 shows the morphology of bacterial pathogens identified from urine cultures out of samples indicated with the 'rods' (Fig. 2B) and 'cocci/mixed' (Fig. 2C) flags. When the UF1000i reports a 'rods' flag for a urine sample, there is a 91% chance that a rod-shaped bacteria is responsible for the positive culture. The remaining 9% are caused by cocci ($N = 16$; 3 are a double infection with a cocci as the main pathogen, but also containing rod-shaped bacteria at a lower concentration). The 'cocci/mixed' flag underperforms, with only 29% of spherical-shaped bacteria (cocci) found in positive cultures ($N = 22$; 58% of cocci classified correctly). In this morphology category the rod-shaped bacteria are again the dominating species with 68%. Besides bacteria, this morphology category also identifies a small percentage of yeast as cocci (3%). As this flag is assigned to 'cocci/mixed' urine samples, the percentage of urines with two or more bacterial species identified was determined. Of the 75 urine samples assigned with a 'cocci/mixed' flag, 11 (15%) were double infections, containing rod-shaped bacteria and cocci. Double infected urines do not get assigned with the 'cocci/mixed' flag exclusively: of the 171 urine samples assigned with a 'rods' flag, 17 were double infected urines (10%), also containing cocci. Table SS1 (Supplementary information) lists all the dominant species found per UF1000i bacterial morphology indication.

4. Discussion

4.1. Evaluating bacterial morphology software

The latest feature on the Sysmex UF1000i was evaluated: its ability to indicate the bacterial morphology. Hereto, the morphology indication was compared to the actual morphology of the bacterial pathogen found in the urine cultures. The urine flow cytometer classifies bacteria into two categories: 'rods' and 'cocci/mixed'. The terminology of the two categories indicates that one category will most likely outperform the other. Indeed, the urine samples assigned with a 'rods' indication, mostly contained rod-shaped bacteria in their cultures (91%). In contrast, the 'cocci/mixed' assigned urines resulted in cultures with a more diverse palette of bacterial pathogen shapes. Only 29% were cocci (58% of all cocci are classified correctly), the large majority (68%) was again rod-shaped.

Chances of finding either rod-shaped bacteria or cocci are not equal. Most UTIs are caused by rod-shaped bacteria, within our hospital population 84% (Fig. 3A; a priori chance 0.84). The post priori chance (ppc) of finding a rod-shaped bacterium, after the 'rods' indication, only marginally increases (from 84% to >91%; ppc = 1.6). The large number of rod-shaped bacteria within the 'cocci/mixed' category is directly responsible for this.

4.2. Foreknowledge on bacterial morphology and antibiotic choice

Foreknowledge of bacterial morphology, could guide the physician in the choice of antibiotic. In general, rod-shaped bacteria are easier to treat, as they lead to uncomplicated UTIs, whereas cocci are responsible for (more) complicated UTIs. In practice, this could lead to a situation where the physician awaits urine screening results and takes the bacterial morphology, bacterial count and clinical symptoms into consideration before deciding on which antibiotic to prescribe. After culture results are available, antibiotic prescription can be switched if needed.

To test if the current antibiotic policy could be changed to an algorithm where the UF1000i morphology indication determines which antibiotic to prescribe, a first-choice antibiotic was selected for all urines assigned with a 'rods' flag (cerfuroxim) and another for those indicated with 'cocci/mixed' (amoxicillin). By using first-choice antibiotics on most samples, one could possibly reduce the increase in antibiotic resistance that bacteria in general, and rods specifically, are infamous for. Most urines contained rod-shaped pathogens (84%, $N = 206$; Fig. 3A), therefore, results are compared to the situation where cefuroxim is prescribed to all patients regardless of morphology indication (Fig. 3B; 72% sensitive, $N = 175$). Fig. 3C depicts the response to cefuroxim of all samples indicated with a 'rods' flag: 75% ($N = 129$) of samples are sensitive, 25% ($N = 42$) are resistant. The resistant samples include both rod- and cocci-shaped bacteria (as indicated by culture results). The response to amoxicillin of samples indicated with a 'cocci/mixed' flag is shown in Fig. 3D. Here 49% ($N = 36$) of samples are sensitive, while 51% ($N = 37$) are resistant. Once again, for both morphologies bacteria are found that are resistant (see also Supplementary information). Preknowledge

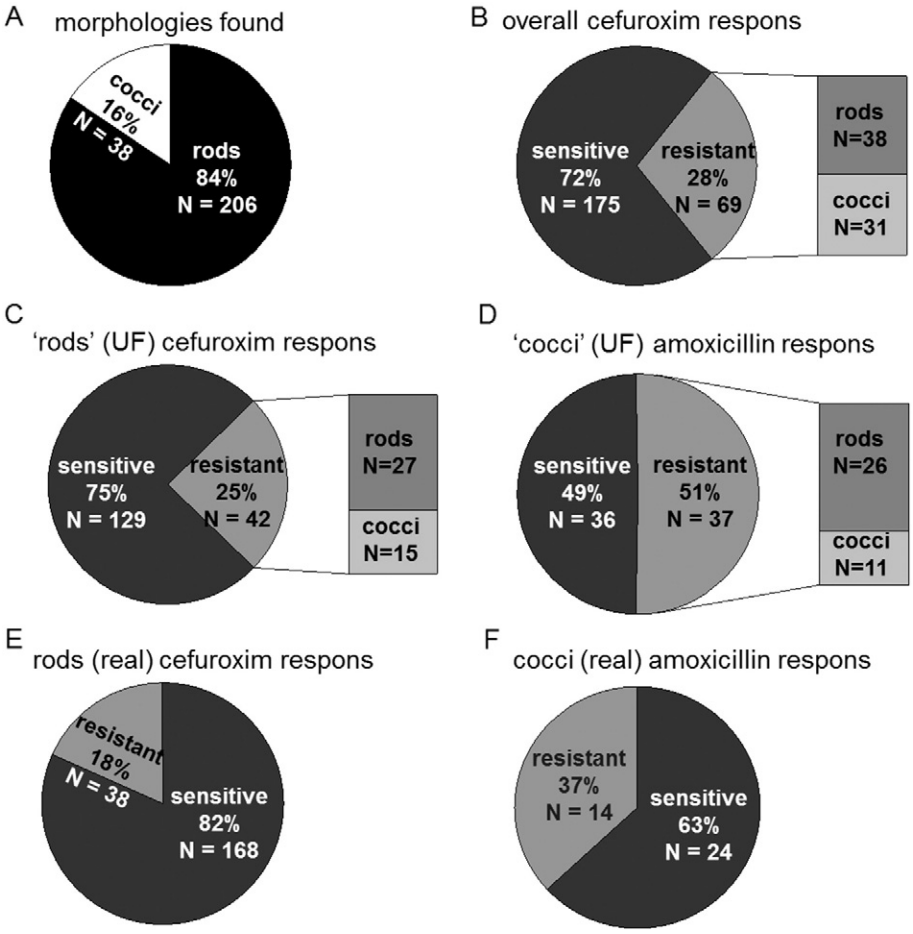

Fig. 3. Response of bacterial pathogens to the first-choice antibiotic per morphology. A. Morphology of the bacterial pathogens found (in cultures). B. Sensitivity to cefuroxim of all pathogens found, regardless of morphology indication by the UF1000i. C. Sensitivity to cefuroxim for all pathogens indicated with a 'rods' morphology flag by the UF1000i. D. Sensitivity to amoxicillin for all pathogens indicated with a 'cocci/mixed' morphology flag by the UF1000i. E. Situation if the rods morphology indicated all rod-shaped bacteria correctly. F. Situation if the cocci/mixed morphology indicated all cocci correct.

of a UF1000i indicated bacterial morphology did not lead to significantly higher percentages of first-choice antibiotic sensitive samples.

For future reference, a scenario where the UF1000i correctly identifies the morphology of all bacteria was also analyzed. When all rod-shaped bacteria are correctly indicated, 82% ($N = 168$) of all rod-indicated samples will be sensitive to cefuroxim (Fig. 3E). The remaining 18% ($N = 38$) cannot be treated with this first-choice antibiotic. Prescribing amoxicillin to those patients with 'cocci/mixed' indicated urine samples, results in an overall sensitivity of 63% (Fig. 3F; $N = 24$). Due to the larger number of rod-infected urines, the overall response (rods and cocci) in this scenario will be 79% ($N = 192$). Not all cocci found are considered true UTI pathogens. For instance, *Staphylococcus aureus* is mostly found in samples from urinary catheters, where it is debatable if the pathogen originates from urine or skin. When only the three unquestionable UTI pathogens (*Streptococcus agalactiae* and both *Enterococci*) are taken into consideration, the sensitivity to amoxicillin increases to 78% ($N = 21$; 81% overall, $N = 189$).

In conclusion, the software upgrade on Sysmex urine flow cytometers is designed to subdivide bacteria into either rod-shaped or cocci. By labeling one category 'rods', while the other is labeled 'cocci/mixed' Sysmex seems to be aware that one outperforms the other. In this study this is clearly shown. Currently, this software upgrade is not good enough to be of use in the clinical setting in our hospital, but this does not mean it cannot develop into a useful tool. Moreover, in a setting with a different population (e.g. smaller percentage of complicated UTIs; for instance general practice or laboratories in other hospitals [12]), the Sysmex bacterial morphology indication may already be of use.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2015.06.020.

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